The MILE study:
Expression microarray analysis for diagnosis of leukaemia

Ken Mills
CCRCB
Queen's University Belfast

CCRCB
Centre for
Cancer Research
& Cell Biology

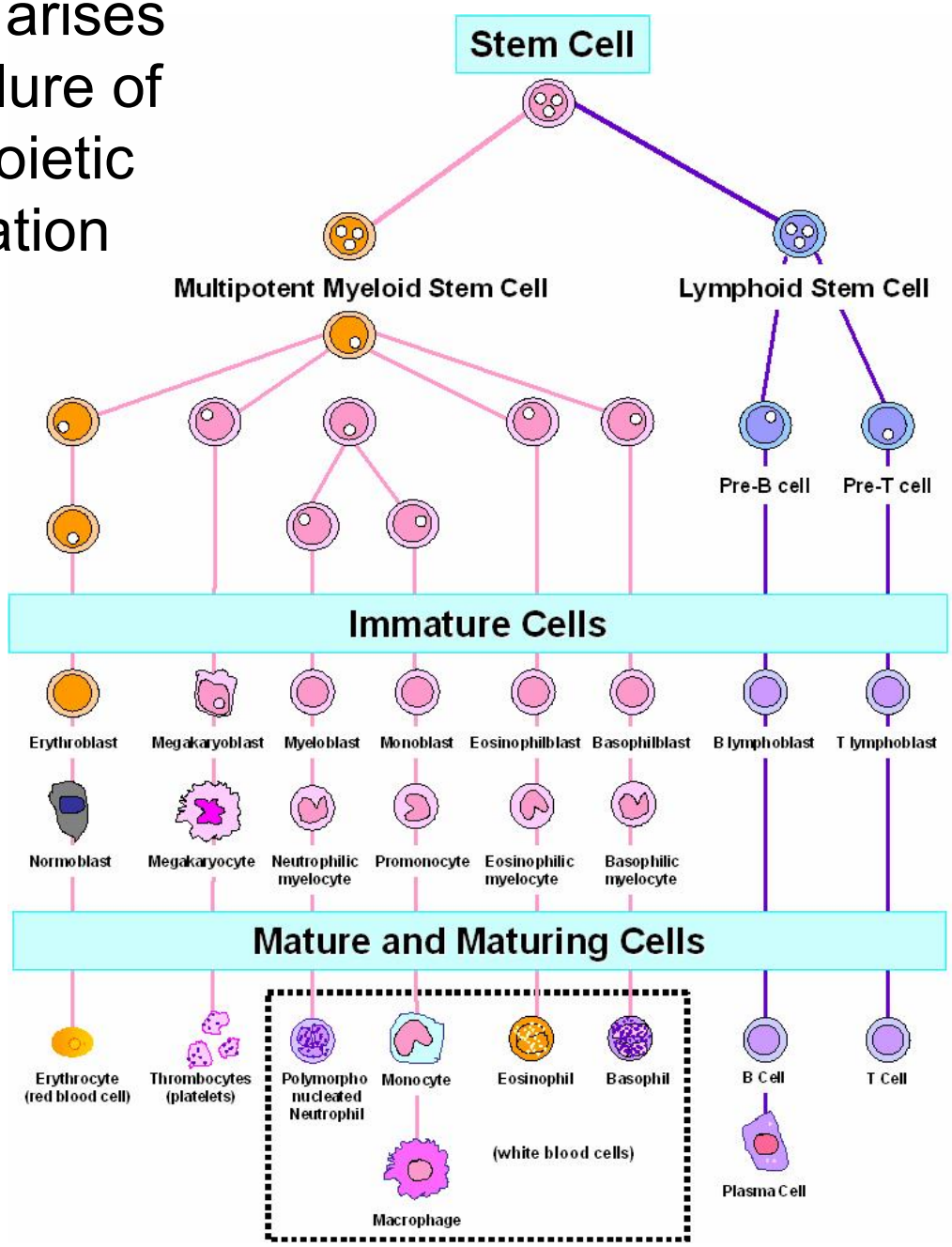
Queen's University
Belfast

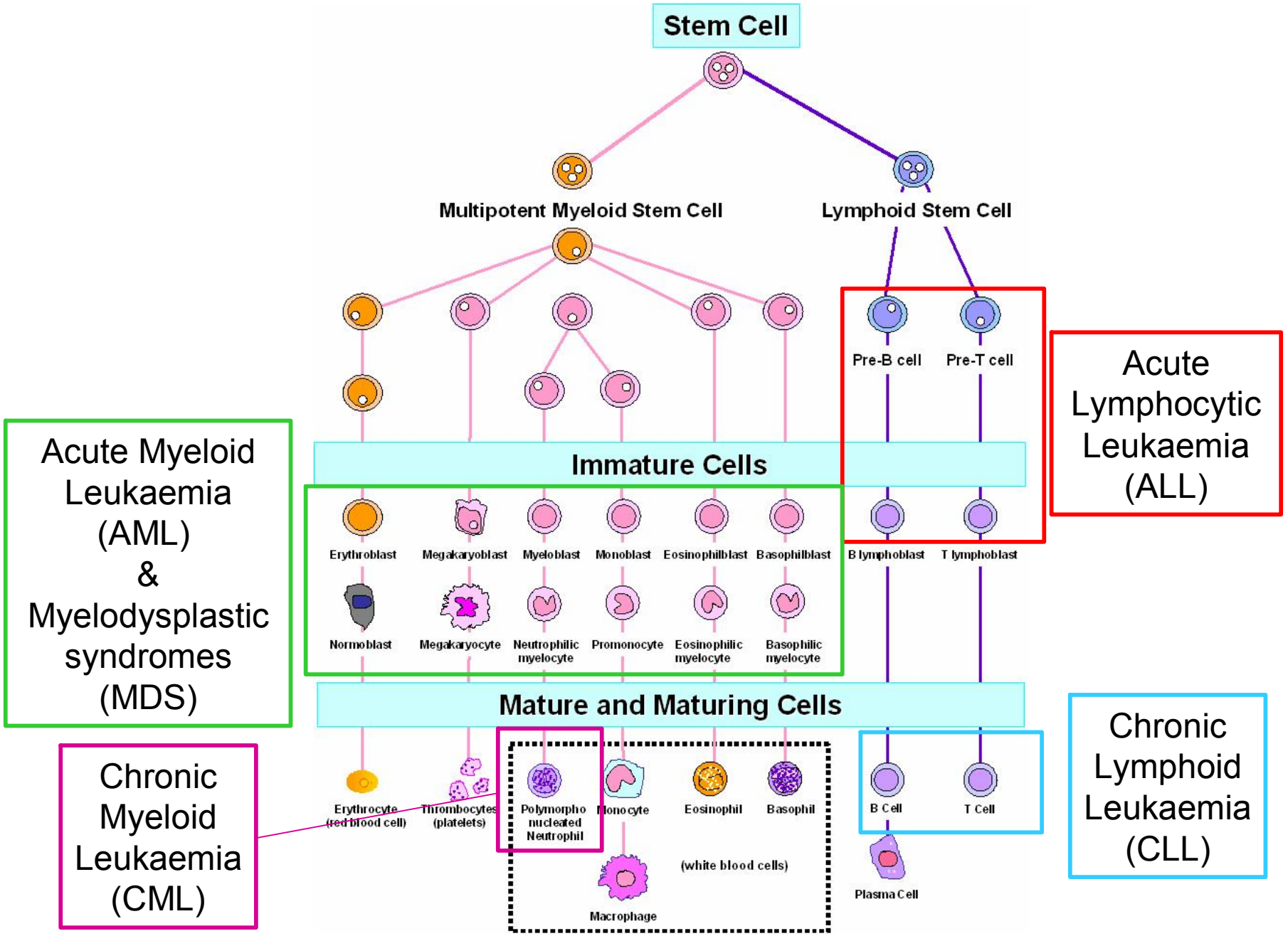
ELN
European
LeukemiaNet

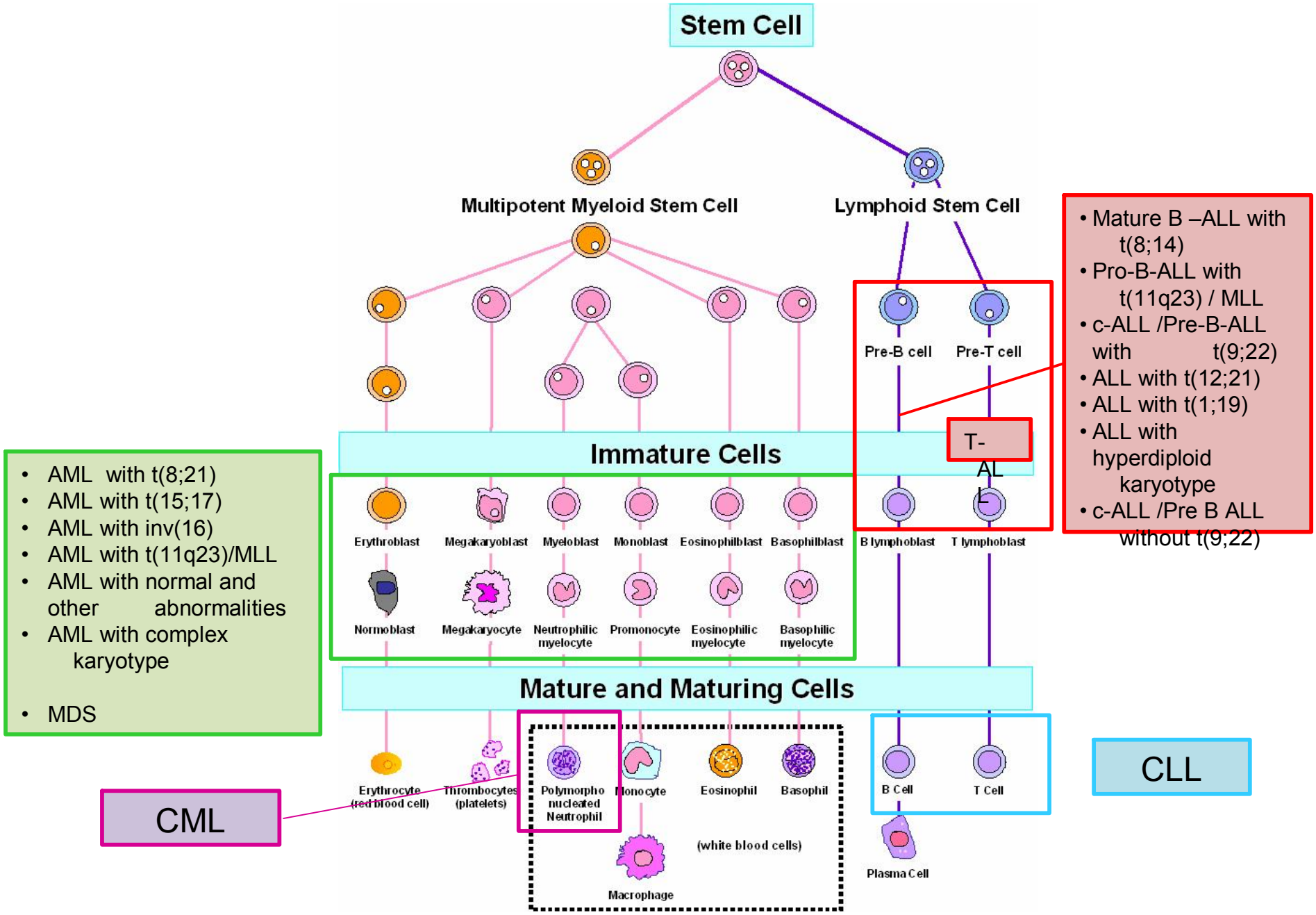
Outline

- MILE
  - Prephase
  - Stage I
  - Stage II
  - Implementation
- MDS
  - Diagnosis into Prognosis

Leukaemia arises due to a failure of haematopoietic differentiation




Stem Cell
Multipotent Myeloid Stem Cell
Lymphoid Stem Cell
Pre-B cell
Pre-T cell
Acute Lymphocytic Leukaemia (ALL)
Immature Cells
Erythroblast
Megakaryoblast
Myeloblast
Monoblast
Eosinophilblast
Basophilblast
B lymphoblast
T lymphoblast
Normoblast
Megakaryocyte
Neutrophilic myelocyte
Promonocyte
Eosinophilic myelocyte
Basophilic myelocyte
Acute Myeloid Leukaemia (AML) & Myelodysplastic syndromes (MDS)
Mature and Maturing Cells
Erythrocyte (red blood cell)
Thrombocytes (platelets)
Polymorpho nucleated Neutrophil
Monocyte
Eosinophil
Basophil
(white blood cells)
Macrophage
B Cell
T Cell
Plasma Cell
Chronic Lymphoid Leukaemia (CLL)
Chronic Myeloid Leukaemia (CML)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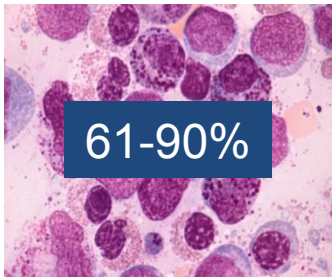
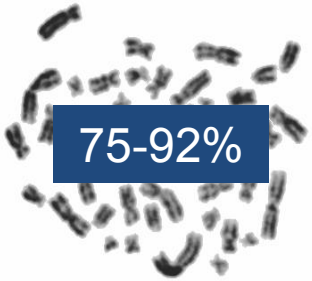
• Mature B –ALL with t(8;14)
• Pro-B-ALL with t(11q23) / MLL
• c-ALL /Pre-B-ALL with t(9;22)
• ALL with t(12;21)
• ALL with t(1;19)
• ALL with hyperdiploid karyotype
• c-ALL /Pre B ALL without t(9;22)
T-ALL
• AML with t(8;21)
• AML with t(15;17)
• AML with inv(16)
• AML with t(11q23)/MLL
• AML with normal and other abnormalities
• AML with complex karyotype
• MDS
CML
CLL

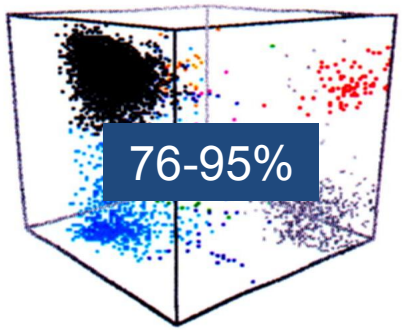
Leukaemia Diagnosis



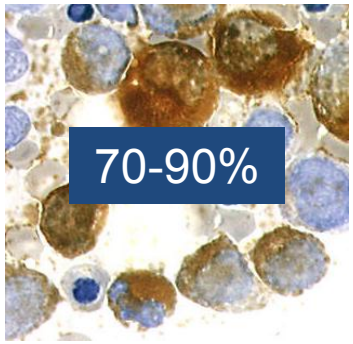
Morphology



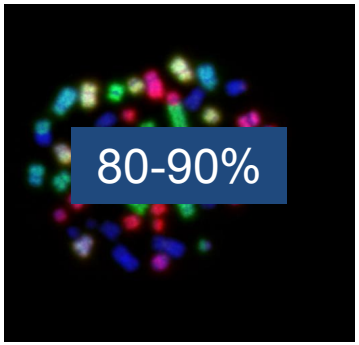
Cytogenetics



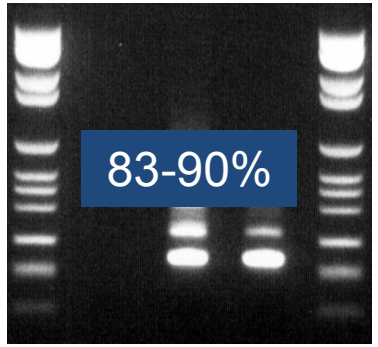
Immunophenotyping



Cytochemistry

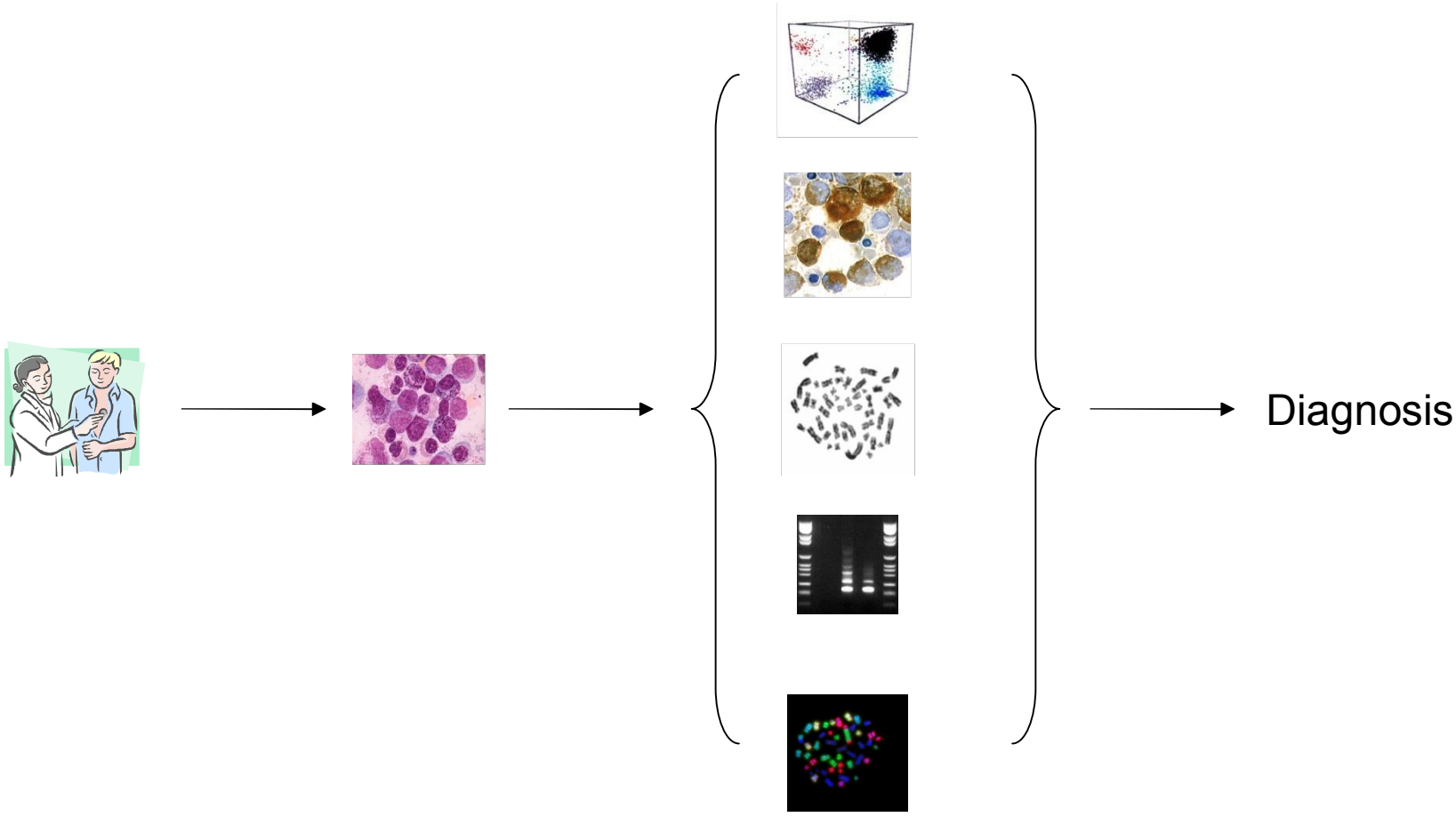


FISH



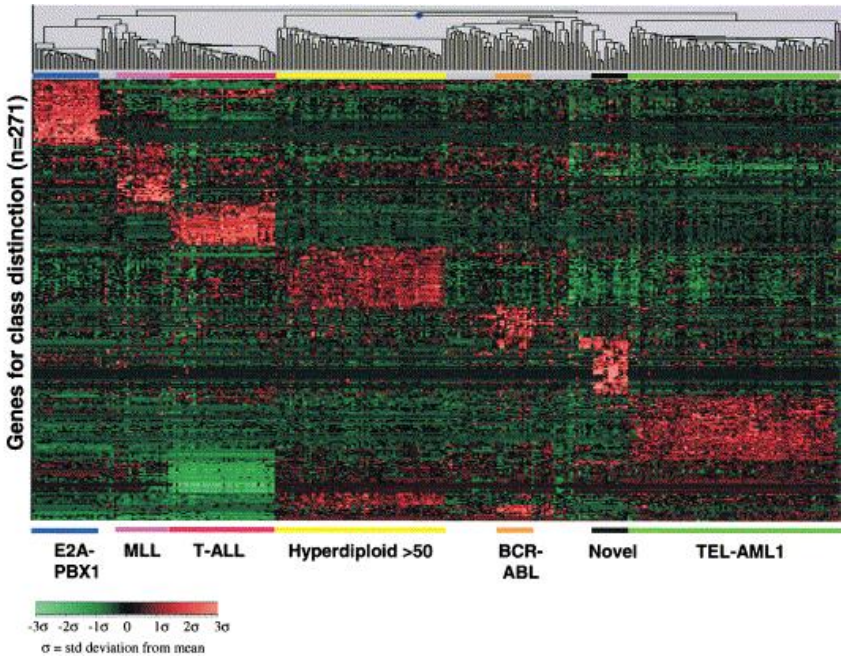
PCR

Leukaemia Diagnosis



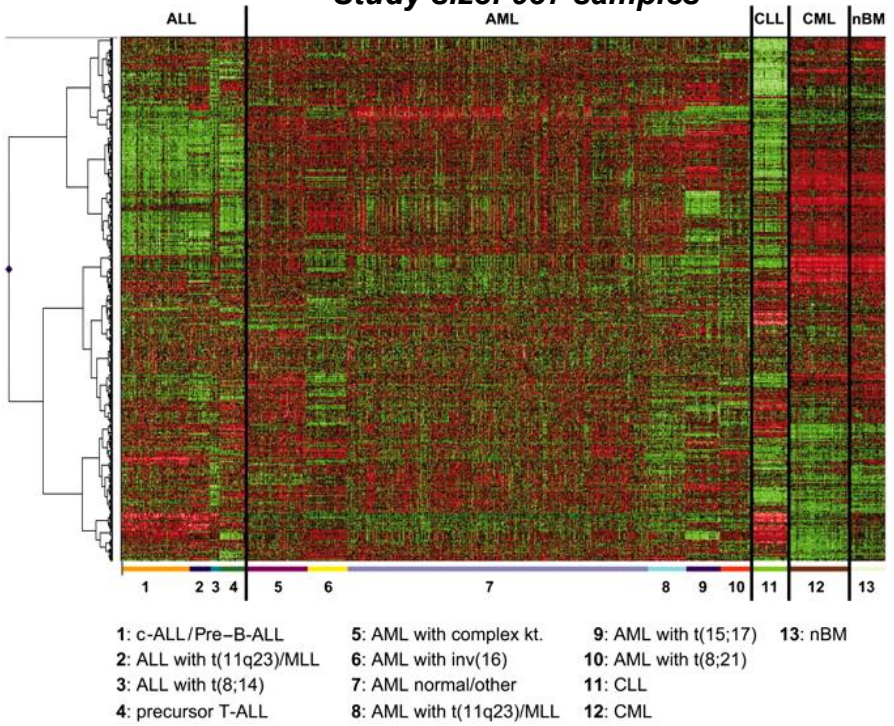
Microarrays identify expression patterns for clinically and therapeutically relevant classes of paediatric and adult leukaemias

Study size: 327 samples



Yeoh et al., Cancer Cell 2002
[Clinically relevant pediatric ALL]

Study size: 937 samples



Haferlach et al., Blood 2005
[Clinically relevant adult leukaemias]

Gene expression profiling has become a powerful and robust technology. The application of microarrays for subclassification of leukaemia subtypes is possible as shown in mono-centric studies.

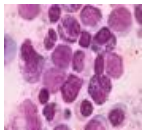
The MILE Study

Microarray Innovations in LEukemia

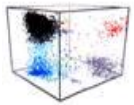
A sequential study to assess the clinical accuracy of the microarray test as compared to standard leukaemia laboratory methods ("Gold Standard") for 16 classes of leukaemia, MDS and non-leukaemia / normal bone marrow

n > 4,000 patients

Gold Standard
Diagnostic Information


Morphology

Cytogenetics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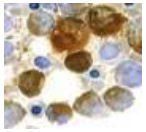
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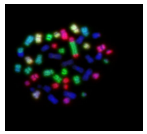
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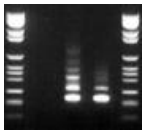
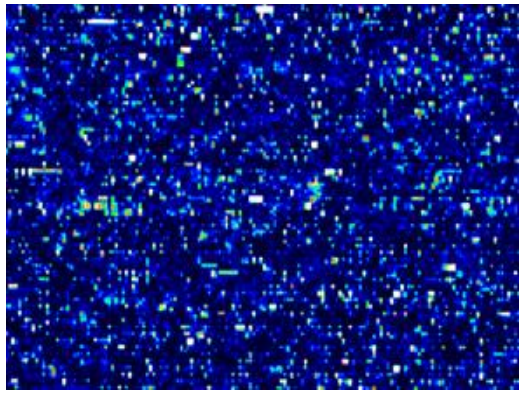
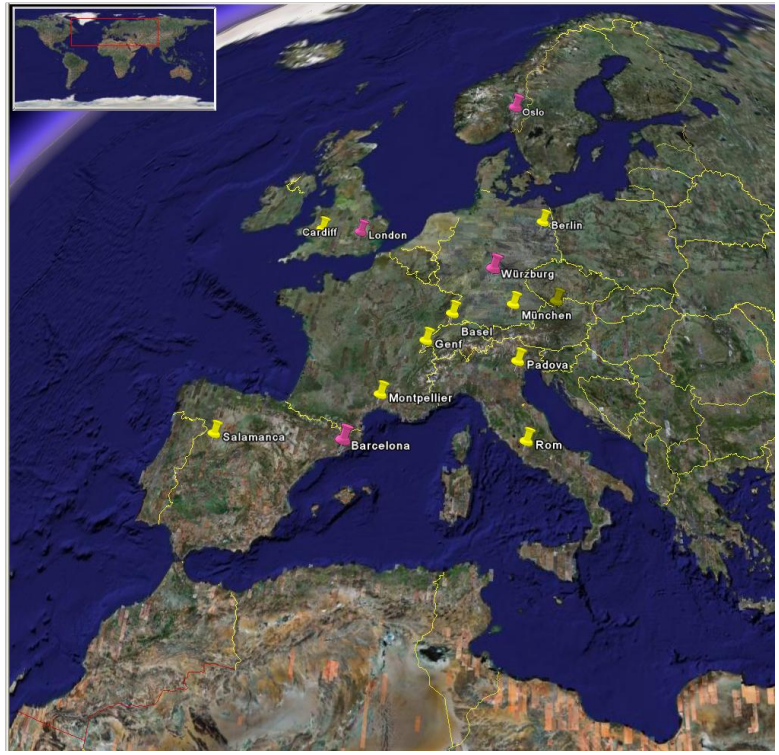

PCR

Microarray-based
gene expression profile



A global biomarker discovery and feasibility program includes 22 centers in 11 countries

Leukaemia (MILE)	**US: 3 centres, EU: 10 centres, Singapore**
Lymphoma (NCI)	**US: 3 centres, EU: 4 centres, Canada**



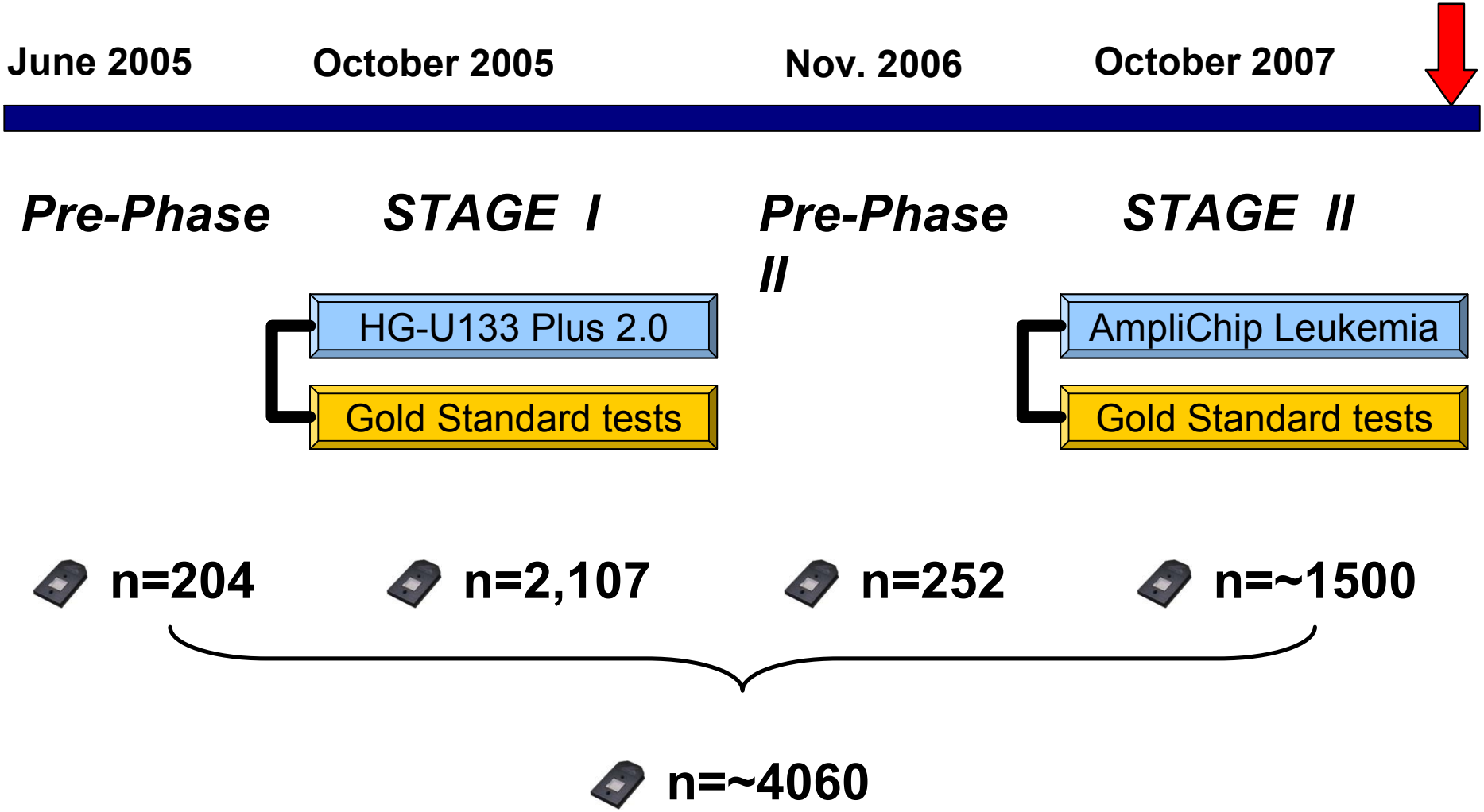
MILE Stage I classification algorithm concept: 16 types of acute, chronic leukaemias, and MDS

No.	Class
1	mature B-ALL with t(8;14)
2	Pro-B-ALL with t(11q23)/MLL
3	c-ALL/Pre-B-ALL with t(9;22)
4	**T-ALL**
5	ALL with t(12;21)
6	ALL with t(1;19)
7	ALL with hyperdiploid karyotype
8	c-ALL/Pre-B-ALL without t(9;22)
9	AML with t(8;21)
10	AML with t(15;17)
11	AML with inv(16)/t(16;16)
12	AML with t(11q23)/MLL
13	AML with normal karyotype + other abnormalities
14	AML complex aberrant karyotype
15	**CLL**
16	CML
17	MDS
18	Non-leukemia and healthy bone marrow

Subclass
ALL, T-lineage, immature (Pre)
ALL, T-lineage, immature (Pro)
ALL, T-lineage, cortical
ALL, T-lineage, mature

Subclass
mutated IgVH
unmutated IgVH
ZAP-70 positive
ZAP-70 negative

MILE Study: Timelines



Pre-phase

Goals of Pre-Phase –

Intra-laboratory reproducibility and inter-laboratory comparability

Each **Operator** is trained on an identical sample preparation protocol

Each **Laboratory** is provided the same laboratory equipment, as well as kits & reagents from the same source for sample preparation and microarray analysis

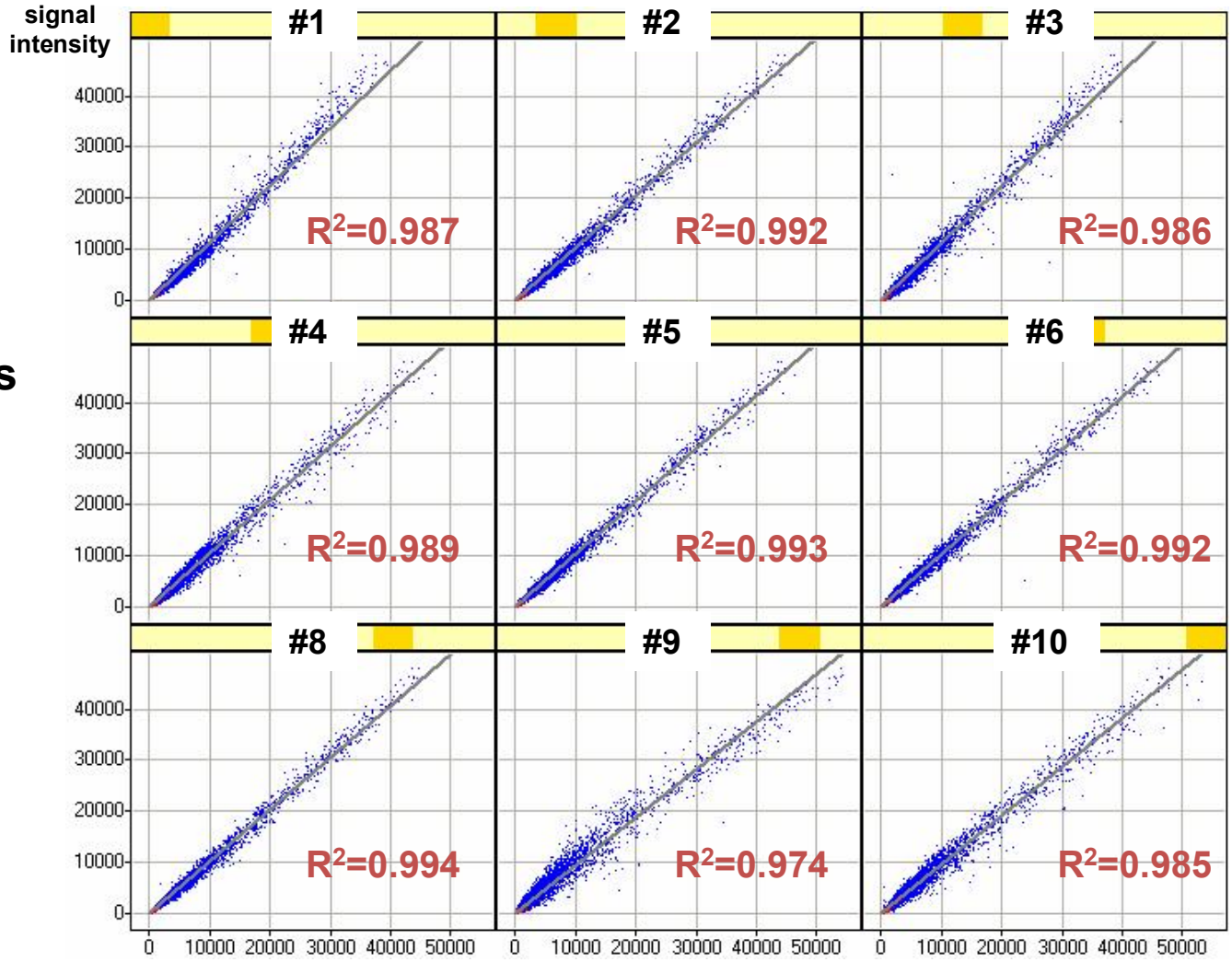
Pre-phase for inter-laboratory reproducibility

Example:

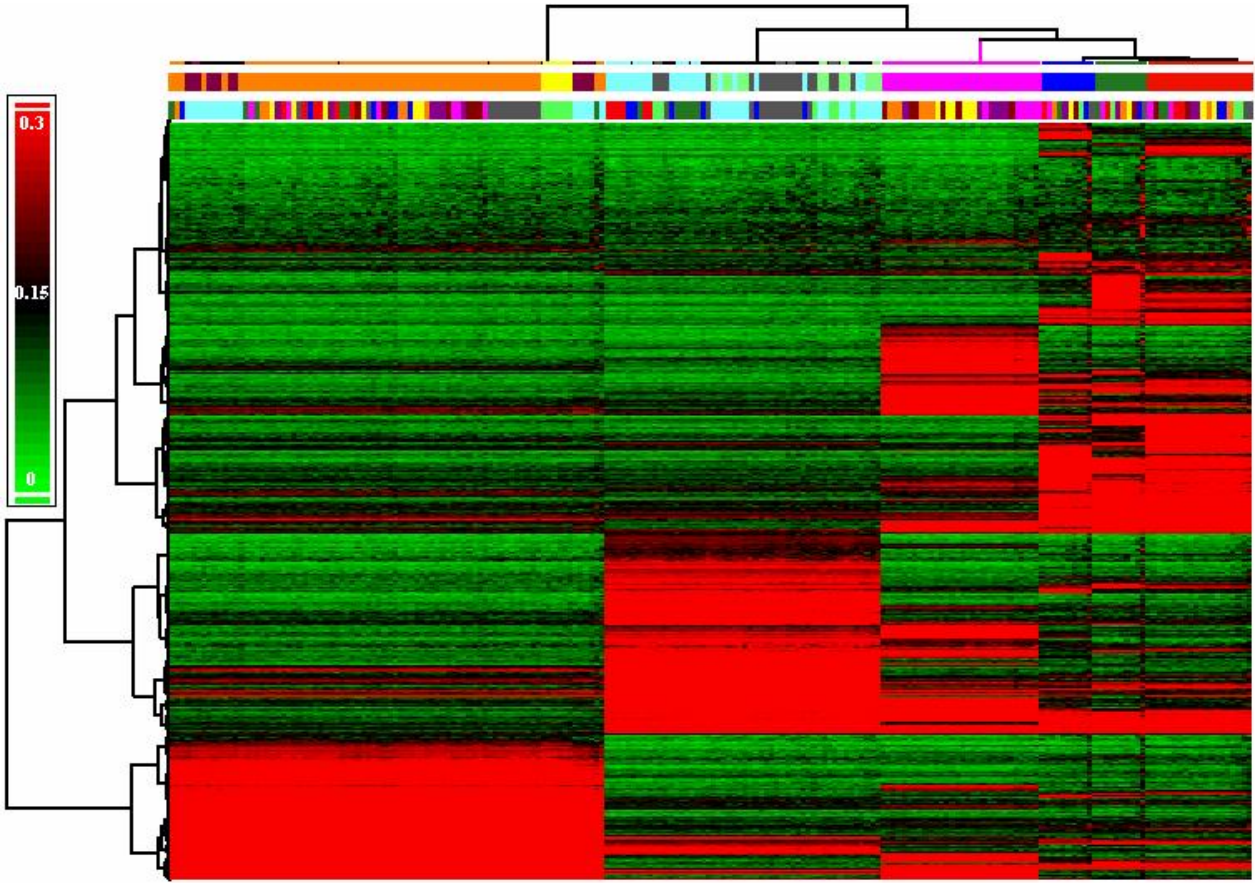
Center 7 vs.

9 other centers

5 µg HEPG2 sample from proficiency testing
(all genes represented on HG-U133 Plus 2.0 microarray)



Pre-phase for inter-laboratory reproducibility



Center Number

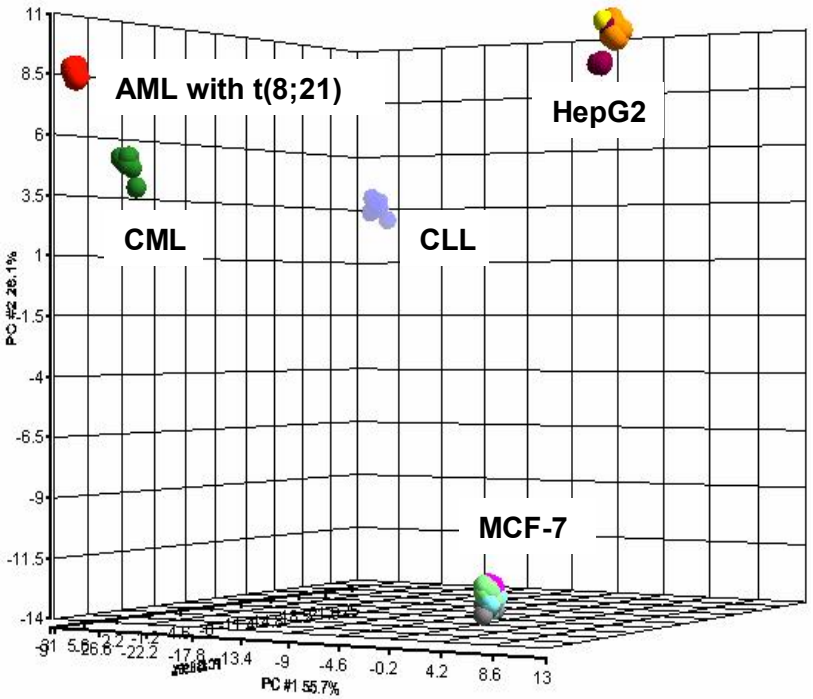
- Site #1
- Site #2
- Site #3
- Site #4
- Site #5
- Site #6
- Site #7
- Site #8
- Site #9
- Site #10
- Site #11

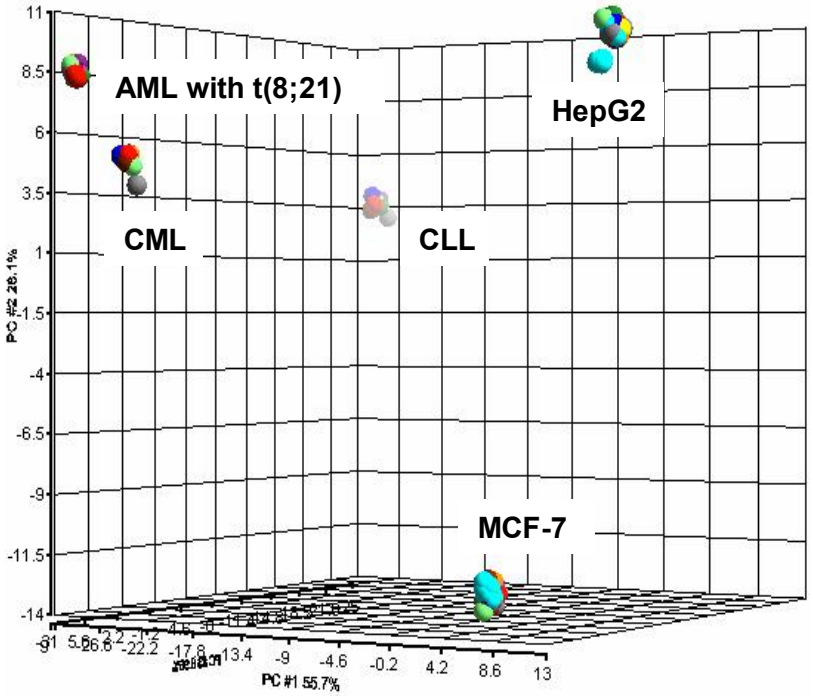
Sample Type

- AML with t(8;21)
- CLL
- CML

- HepG2 (Lot #1)
- HepG2 (Lot #2)
- HepG2 (Lot #3)
- MCF-7 (Lot #1)
- MCF-7 (Lot #2)
- MCF-7 (Lot #3)
- MCF-7 (Lot #4)

Pre-phase for inter-laboratory reproducibility





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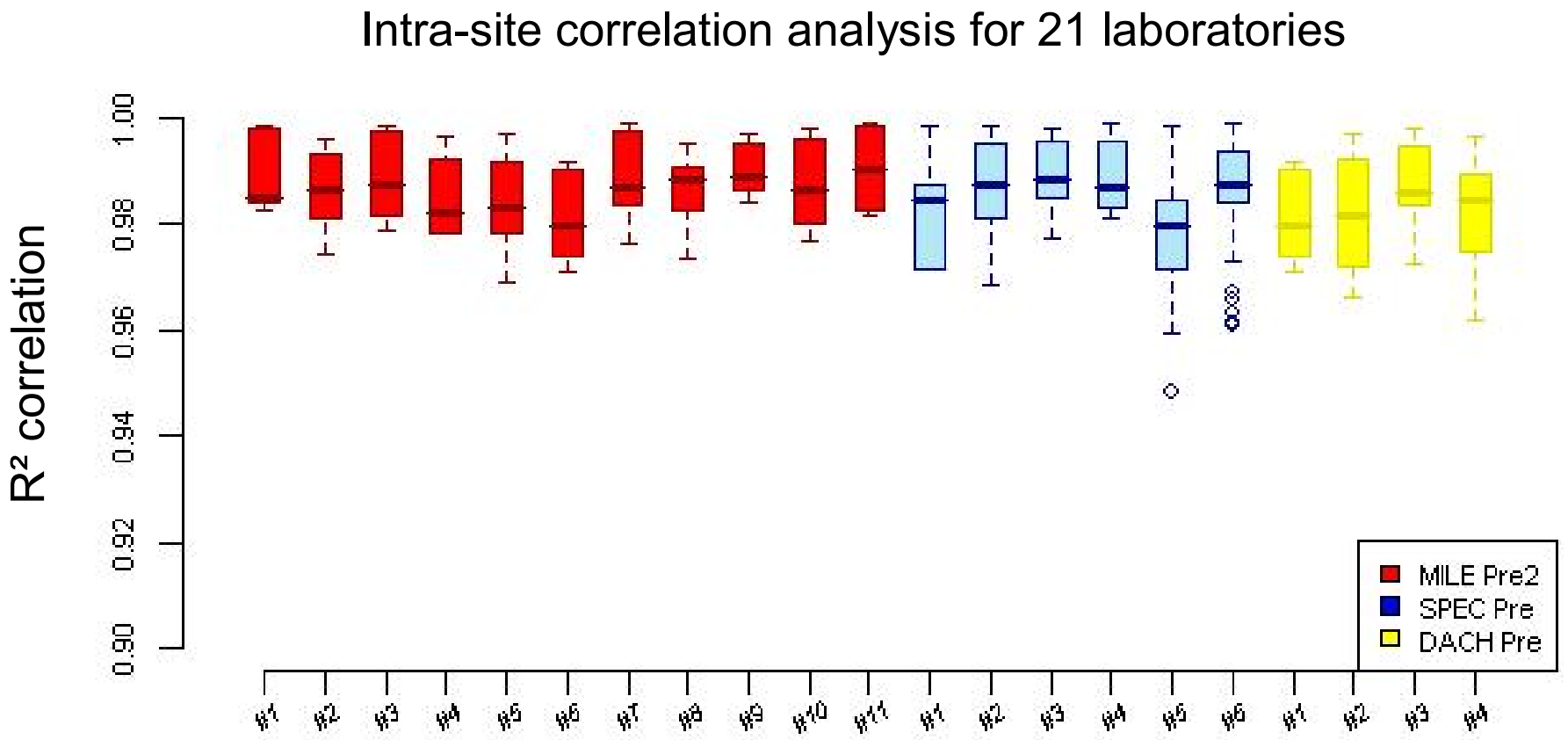
Leukaemia and Lymphoma Proficiency Data

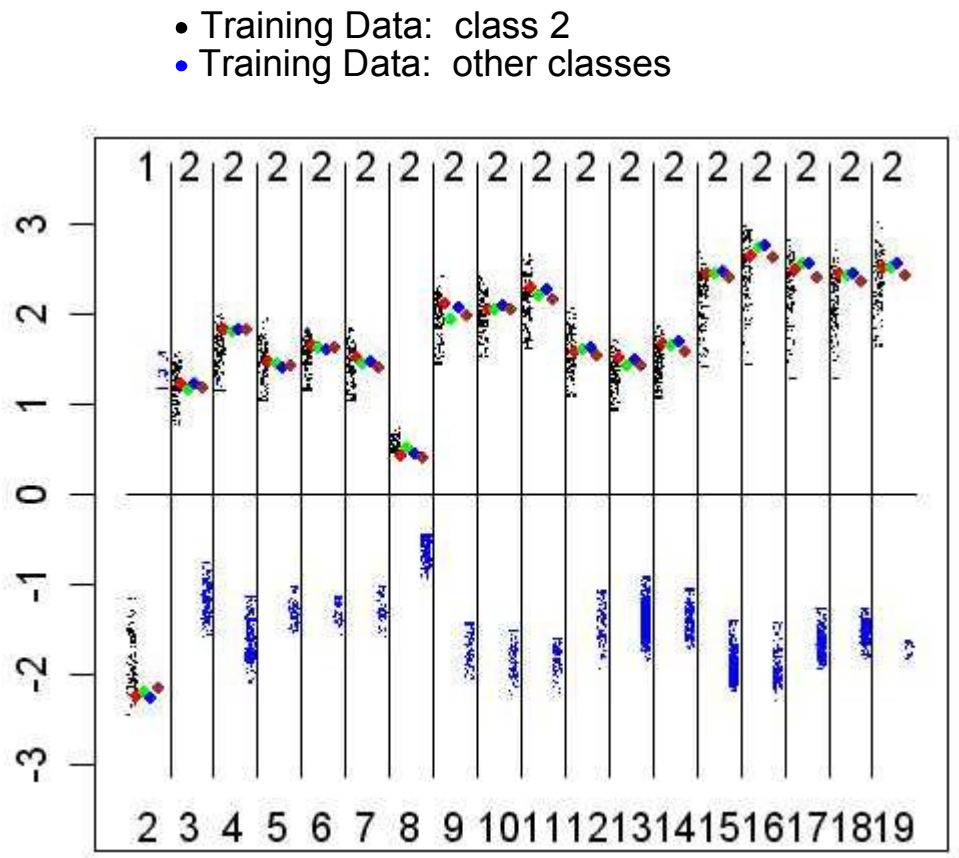


Data is shown for the HepG2 cell line (all genes represented on the Plus 2.0 array)

Intra-laboratory comparison of gene expression values from HepG2 replicates

Leukaemia Classifier – Version 7 (LCv7)

- Model 7 is an all-pair-wise linear classification model for 18 classes (16 leukaemia classes, MDS, and none-of-the-targets)
- Consists of 18 * (18 – 1) / 2 = 153 classifiers
- Uses 534 probe sets on HG-U133 Plus 2.0 microarray



ID: 07-008434 (c2)

Stage I

Data Set (2,916 whole genome profiles)

Class		Sensitivity	Specificity
1	mature B-ALL with t(8;14)	0.85	1.00
2	Pro-B-ALL with t(11q23)/MLL	1.00	1.00
3	c-ALL/Pre-B-ALL with t(9;22)	0.90	1.00
4	T-ALL		1.00
5	ALL with t(12;21)		1.00
6	ALL with t(1;19)		1.00
7	ALL with hyperdiploi		1.00
8	c-ALL/Pre-B-ALL wi		0.99
9	AML with t(8;21)		1.00
10	AML with t(15;17)		1.00
11	AML with inv(16)/t(1		1.00
12	AML with t(11q23)/MLL	0.92	1.00
13	AML with normal karyotype + other abnormalities	0.93	0.99
14	AML complex aberrant karyotype	0.90	1.00
15	CLL	1.00	1.00
16	CML	0.97	1.00
17	MDS	0.88	0.98
18	*None of the above*	0.99	0.99

Accuracy by cross-validation:

93.9%

- based on 30-fold CV
- all HG-U133 Plus 2.0

Stage I

Data Set (2,662 whole genome profiles excluding MDS)

Class		Sensitivity	Specificity
1	mature B-ALL with t(8;14)	0.85	1.00
2	Pro-B-ALL with t(11q23)/MLL	1.00	1.00
3	c-ALL/Pre-B-ALL with t(9;22)	0.90	1.00
4	T-ALL		1.00
5	ALL with t(12;21)		1.00
6	ALL with t(1;19)		1.00
7	ALL with hyperdiploi		1.00
8	c-ALL/Pre-B-ALL wi		0.99
9	AML with t(8;21)		1.00
10	AML with t(15;17)		1.00
11	AML with inv(16)/t(1		1.00
12	AML with t(11q23)/MLL	0.92	1.00
13	AML with normal karyotype + other abnormalities	0.93	0.99
14	AML complex aberrant karyotype	0.90	1.00
15	CLL	1.00	1.00
16	CML	0.97	1.00
18	*None of the above*	0.99	0.99

Accuracy by cross-validation:

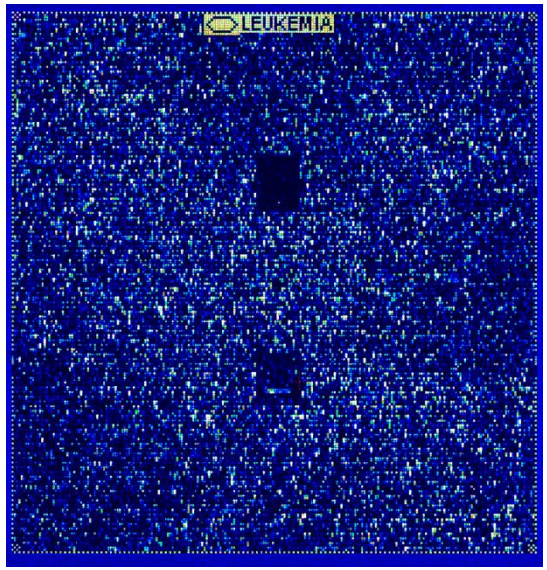
95.3%

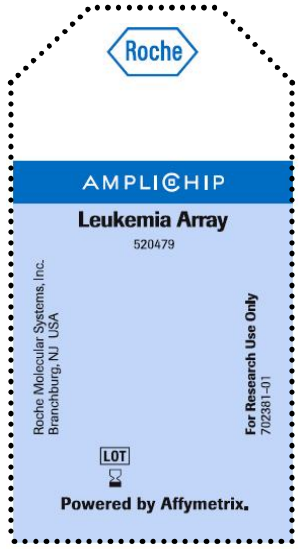
- based on 30-fold CV
- all HG-U133 Plus 2.0

Stage II

- Class 19 introduced
  - Normal PB
  - Class 18: normal BM)
- Prospective samples obtained
- Amplichip used for analysis
  - ~1100 probe sets
  - 539 probe sets for classification
  - CD markers
  - Normalisation and control probe sets
  - Repeated 4 time across chip
- Centre-blinded array result
- QC checked
  - Lower QC failure rate than Stage I
- Concordance determined
- Discordant results checked
  - Array call confirmed
  - Array call not confirmed

Customized design:
AmpliChip Leukaemia





Standardized workflow for routine clinical laboratory usage (< 2.5 h hands-on time)

previous kit configuration

Primer + Controls + Buffer

Mix components & Enzymes

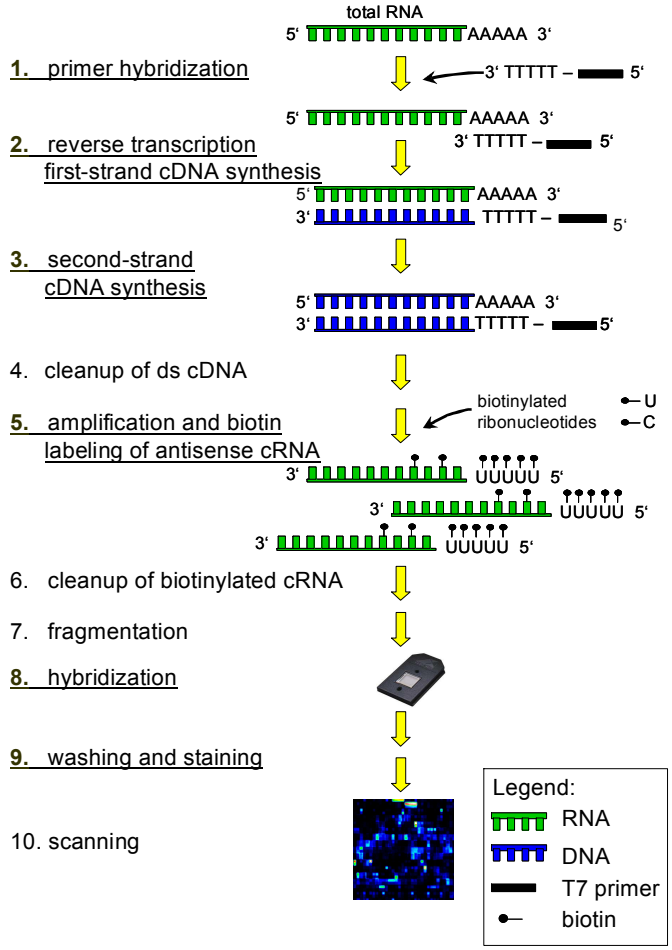
Mix components & Enzymes

Mix components & Enzymes

Mix

Mix components

Stain 1 & 2 components



reduced complexity with final kit

Primer/ Control Mix

Mix & Enzymes

Mix & Enzymes

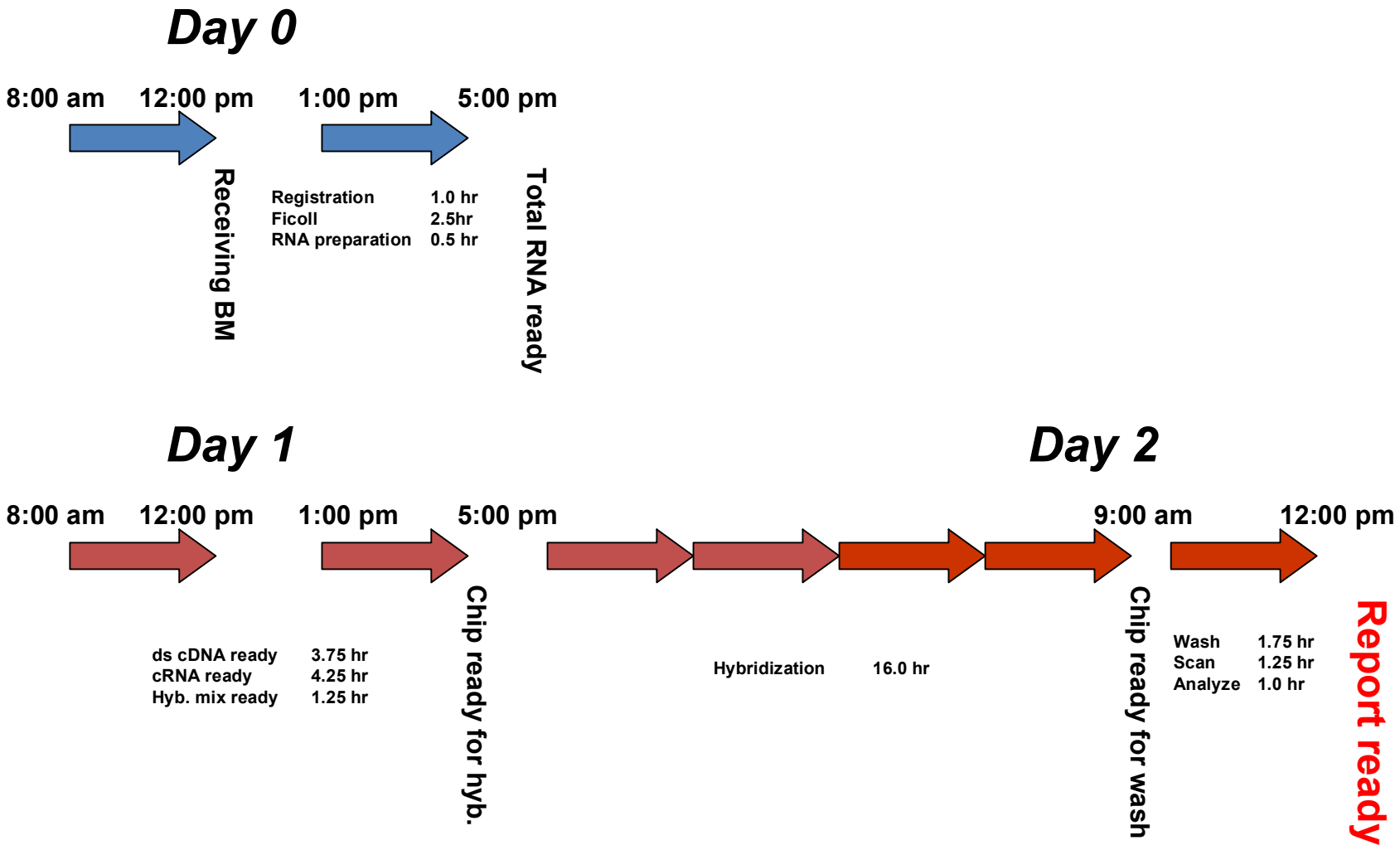
Mix & Enzymes

Mix

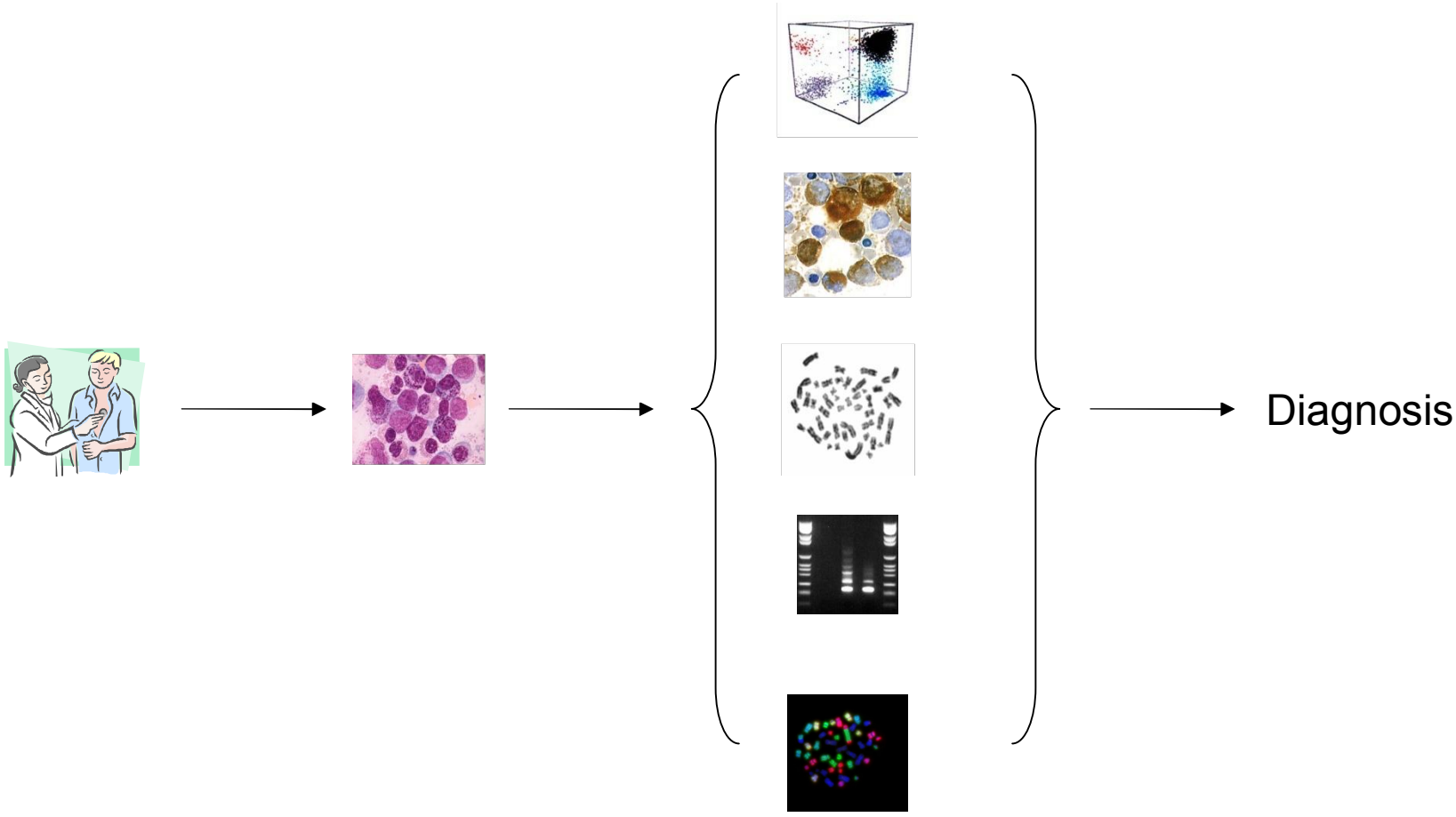
Mix

Stain 1 & Stain 2

Laboratory workflow concept for prospective specimen collection: Results in < 48 hours



Leukaemia Diagnosis



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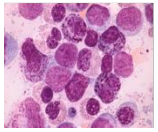
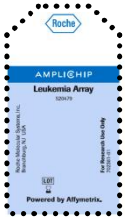
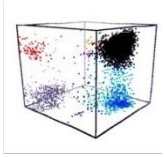
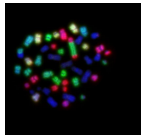
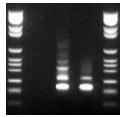






Interim Diagnosis

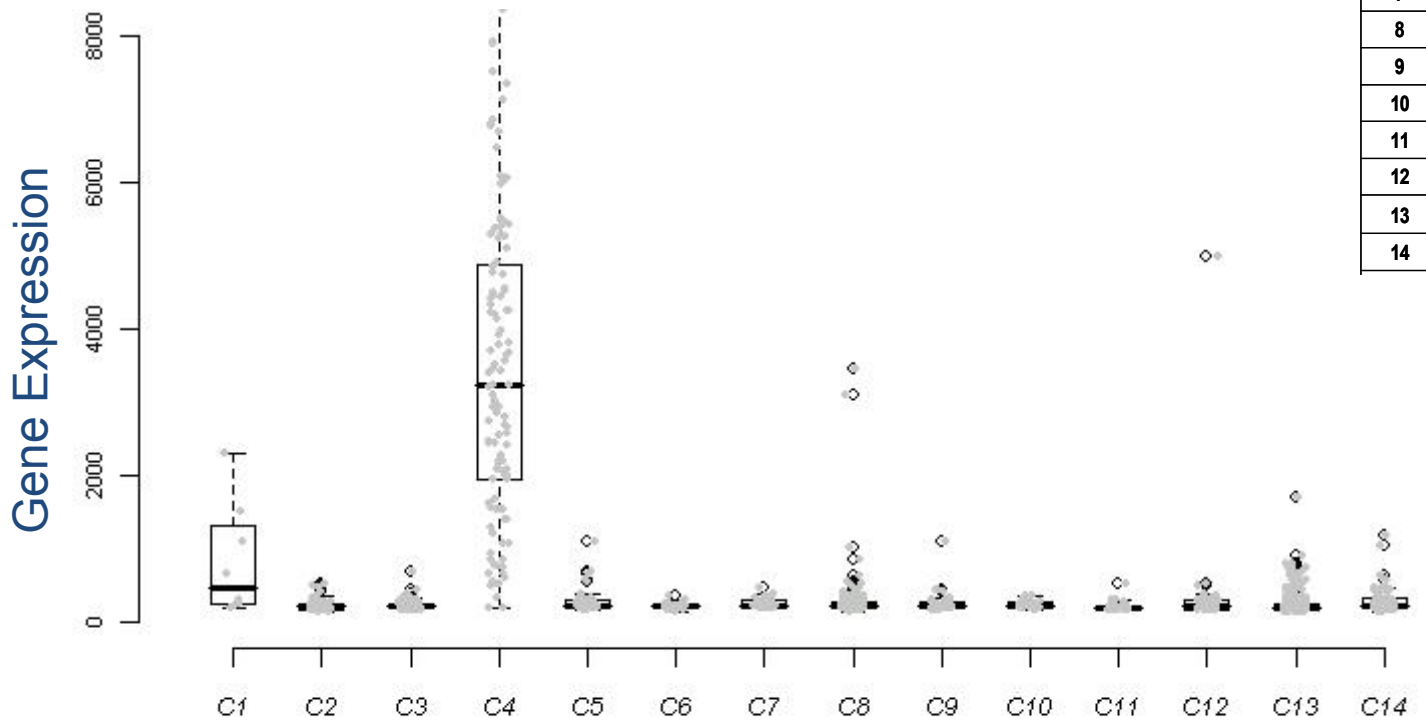




Diagnosis

“Virtual Immunophenotyping”

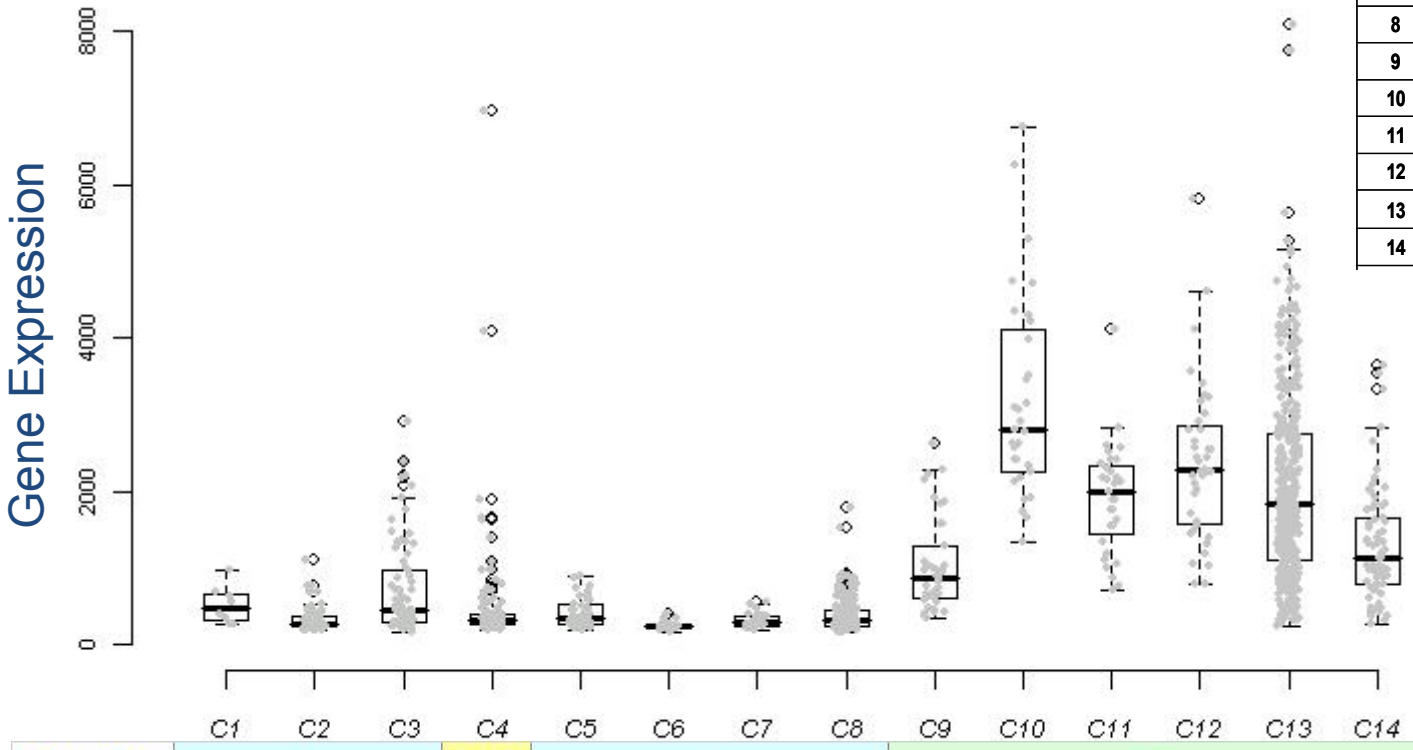
1	mature B-ALL with t(8;14)
2	Pro-B-ALL with t(11q23)/MLL
3	c-ALL/Pre-B-ALL with t(9;22)
4	T-ALL
5	ALL with t(12;21)
6	ALL with t(1;19)
7	ALL with hyperdiploid karyotype
8	c-ALL/Pre-B-ALL without t(9;22)
9	AML with t(8;21)
10	AML with t(15;17)
11	AML with inv(16)/t(16;16)
12	AML with t(11q23)/MLL
13	AML with normal karyotype + other abnormalities
14	AML complex aberrant karyotype



Leukemia Type	B-ALL			T-ALL	B-ALL				AML					
N	8	62	76	116	41	25	32	163	37	32	31	40	359	69
%P	63%	18%	24%	97%	54%	4%	28%	30%	22%	16%	16%	20%	22%	39%
Mean	824.8148	237.0229	235.0884	3447.732	307.3067	224.1451	259.2421	303.223	270.3386	244.0073	224.28	367.2348	255.1604	296.3412
StdDev	768.1744	77.79264	72.51045	2063.485	177.5311	42.27795	69.52565	351.0806	155.9649	59.49919	70.50167	753.2512	145.1394	179.2288

“Virtual Immunophenotyping”

CD33



Leukemia Type	B-ALL			T-ALL	B-ALL				AML					
N	8	62	76	116	41	25	32	163	37	32	31	40	359	69
%P	38%	19%	55%	22%	34%	4%	13%	27%	81%	100%	100%	100%	93%	86%
Mean	512.7822	338.206	728.6859	502.1622	416.523	246.6746	313.9156	397.2391	1040.271	3179.484	1909.896	2380.001	2038.148	1291.595
StdDev	245.8416	169.8528	613.2069	754.2517	194.8763	53.41984	86.54727	229.8592	617.3886	1320.053	716.9842	1032.686	1229.195	748.5669

1	mature B-ALL with t(8;14)
2	Pro-B-ALL with t(11q23)/MLL
3	c-ALL/Pre-B-ALL with t(9;22)
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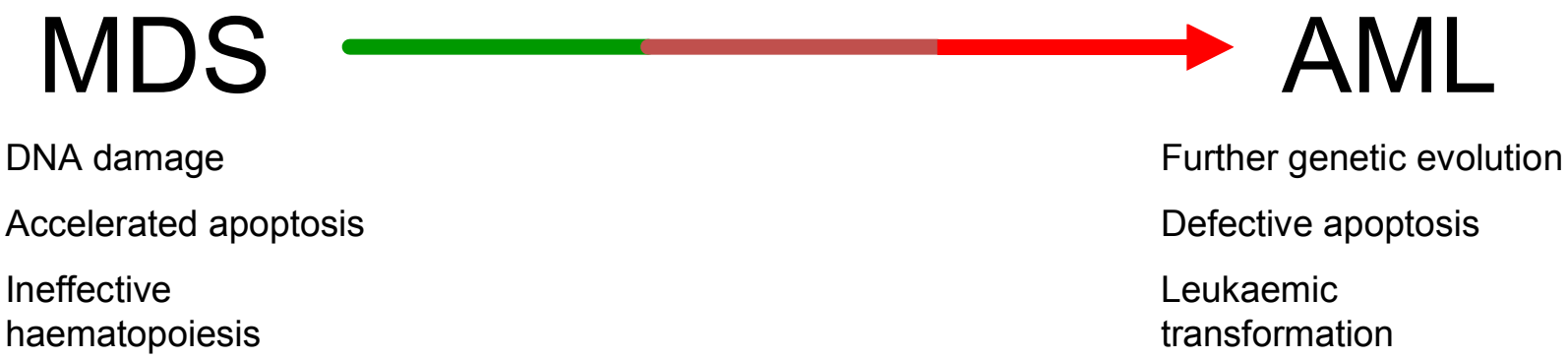
14	AML complex aberrant karyotype

MDS and AML - Relationship

Are AML and MDS part of a continuous spectrum of disease and not specific disorders?

MDS is a pre-leukaemic disorder in which the neoplastic clone has been established, but which may or may not fully progress to AML



MILE study Stage I
MDS:

- 174 MDS samples analysed
  - Berlin
  - Cardiff
  - Munich
  - Salamanca
- 49.1% correctly called **MDS**
  - (Class 17)
- 24.6% called “AML”
  - 20.0% AML with normal or others (Class 13)
  - 4.6% AML with complex cytogenetics (Class 14)
  - **“MDS with an AML like signature”**
- 24.0% called Non-leukaemia / Normal bone marrow
  - (Class 18)
  - **“MDS with a non-leukaemia like signature”**

External Morphology Review

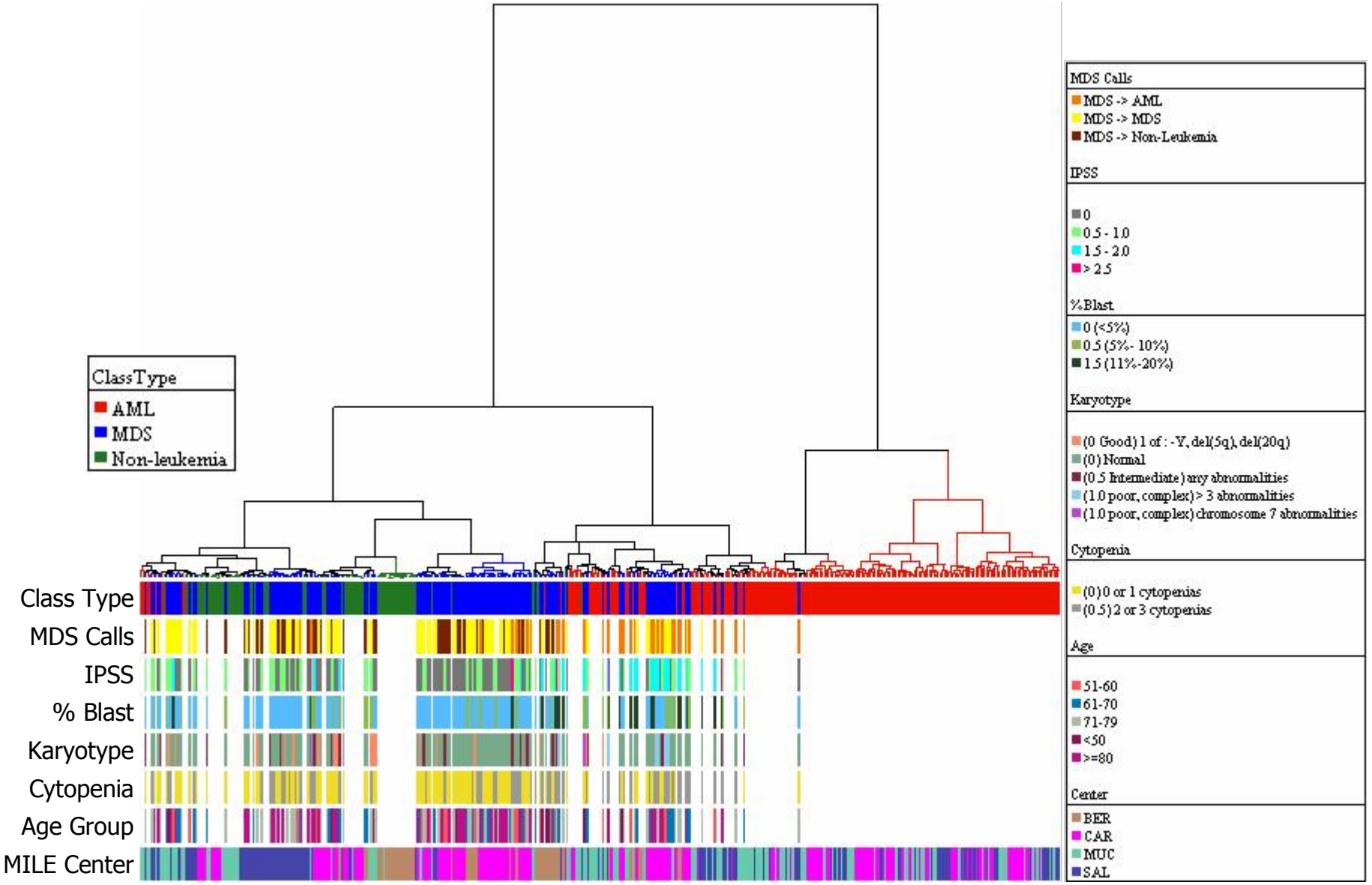
- Morphology slides for submitted MDS samples reviewed by:
  - Prof Loeffler; Germany
  - Dr Bowen; UK
- Review of slides included:
  - FAB
  - WHO
  - Blast count
  - Cytopenia

164 confirmed as MDS and assigned a WHO classification

10 discordant samples removed from MDS analysis

- 6 Reclassified as AML
- 4 Excluded from MDS & MILE study

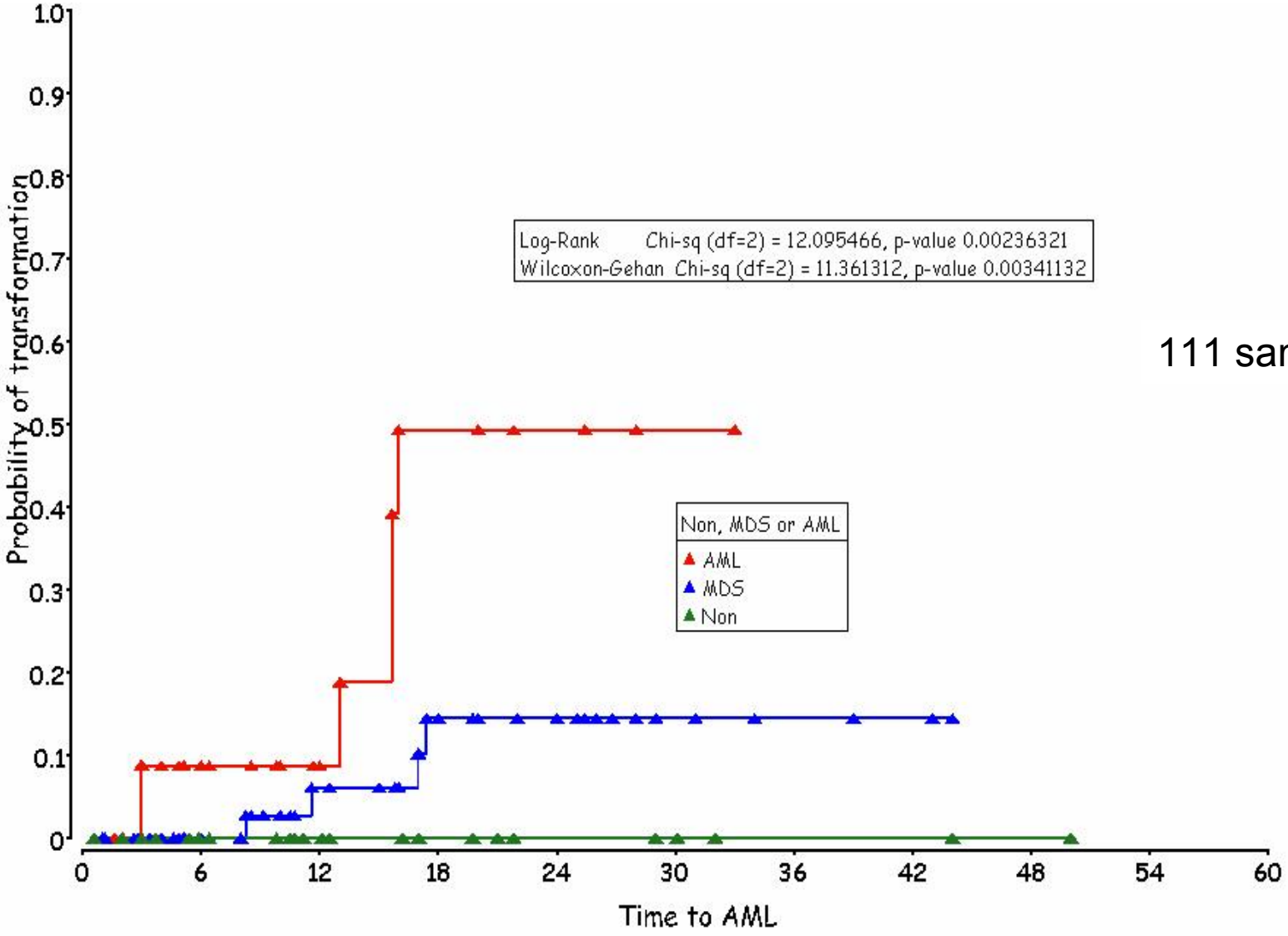
  aCML, CLL, myeloma and incomplete diagnosis



436 samples: 203 AML, 164 MDS, 69 Non-leukaemia

I.C.v.7: 534 probe sets: Euclidean & Ward's

Transformation to AML



111 samples

MILE Study

- High accuracy of disease classification over 16 classes of leukaemia
- High degree of inter- and intra-laboratory correlation
- Rapid result - <48 hrs
- Cost comparable to existing diagnostic tests
  - Refined workflow
- Adaptable to include new validated disease classifications
- Possible prognostic implications

Acknowledgements

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- Germany
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  - Salamanca: Jesus Hernandez
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  - Nancy: Marie Christine Bene
  - Paris: Elizabeth McIntyre
  - Monpellier: Tom de Vos
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  - Cardiff: Alan Burnett

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